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Ultrastructure of the Intestinal Epithelium, Lumen, and Associated Bacteria in *Heterorhabditis bacteriophora*[1]

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ABSTRACT: *Heterorhabditis bacteriophora* is an obligate parasite of insects that contains the bacterial symbiont, *Xenorhabdus luminescens,* in its intestinal lumen. Ultrastructural observations were made of the intestinal epithelium, lumen, and associated bacteria. The oligocytous intestine of the infective juvenile of *H. bacteriophora* has junctional complexes that delineate the boundaries of cells forming the intestinal lumen. Fine structure of the intestinal cells includes microvilli on the apical membranes bordered by a dense matrix of vesicular endoplasmic reticulum and accumulations of variably stained electron-dense granules within each cell. Rod-shaped bacterial cells occur singly or en masse in the lumen of the intestine. Some bacteria appear to undergo autolysis or show interaction with the lumen contents.

KEY WORDS: ultrastructure, *Heterorhabditis bacteriophora,* Nematoda.

Current interest in developing alternatives to the use of chemicals for control of agricultural pests focuses attention on nematode species that can be used for biological control (Gaugler, 1987; Ghally, 1987; Gaugler et al., 1989; Kondo and Ishibashi, 1989; Figueroa and Roman, 1990; Glazer and Wysoki, 1990; Nickle and Cantelo, 1991). Among such species are nematodes, harboring bacteria within their intestinal lumen, that serve as vectors of potential toxicants to insects after host tissue penetration (Dutky and Hough, 1955; Dutky, 1959; Poinar, 1979). Bacteria have been observed in the lumen of the pharynges and intestines of nematodes, and in a certain species, in the epithelial cells forming the lumen (Poinar, 1966; Poinar and Leutenegger, 1968; Poinar et al., 1977). Microvilli, which are a consistent feature of intestinal epithelia of these nematodes, occur on the apical membrane of intestinal cells. Among the animal parasitic nematodes, the brush border of the infective larvae of *Trichinella spiralis* was shown to consist of microvilli (Bruce, 1966). Similarly, an ultrastructural study of the intestine of *Capillaria hepatica* showed microvilli occurring on the epithelial surfaces (Wright, 1963). Microvilli have been found and described in a wide range of nematode species that include animal, insect, and plant–parasitic species (Wright, 1963; Sheffield, 1964; Shepherd and Clark, 1976; Munn and Greenwood, 1984; Endo, 1988).

With a goal to establish criteria for evaluating the effectiveness of nematodes as biocontrol agents of insects such as the wax moth, *Galleria mellonella* (L.), observations were made on the fine structure of the intestinal epithelia of *Heterorhabditis bacteriophora* Poinar (1975). Emphasis was on: (1) cell morphology and its components, (2) surface membrane modifications related to microvilli formation, and (3) distribution of associated bacteria, *Xenorhabdus luminescens* Thomas and Poinar, 1979, in the intestinal lumen.

Materials and Methods

Dauer larvae of *Heterorhabditis bacteriophora* from a culture maintained by Biosys, Inc. in an alginate gel were released in water. The infective juveniles (J3) were then concentrated and suspended in water agar prior to fixation and embedded according to previously published procedures (Endo and Wergin, 1973; Endo, 1984, 1987). Briefly, nematodes in a suspension of water were mixed with warm liquefied 2% water–agar and the mixture was poured into small grooves in agar-filled petri dishes. The solidified agar, containing the nematodes, was diced into 2–3-mm blocks that were transferred to glass vials containing 3% glutaraldehyde in 0.05 M phosphate buffer (pH 6.8) at 22°C for chemical fixation of the larvae. Subsequent rinsing and postfixation in osmium tetroxide were also carried out in 0.05 M phosphate buffer (pH 6.8). The glutaraldehyde fixation (for 1.5 hr) was followed by washing in 6 changes of buffer over a period of 1 hr. The agar blocks were then postfixed in 2% osmium tetroxide for 2 hr at 22°C, dehydrated in an acetone series and infiltrated with a low viscosity embedding medium (Spurr, 1969). Silver-gray sections of selected nematodes were cut with a dia-

[1] Mention of a trade name, warranty, proprietary product, or vendor does not constitute a guarantee of a product and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

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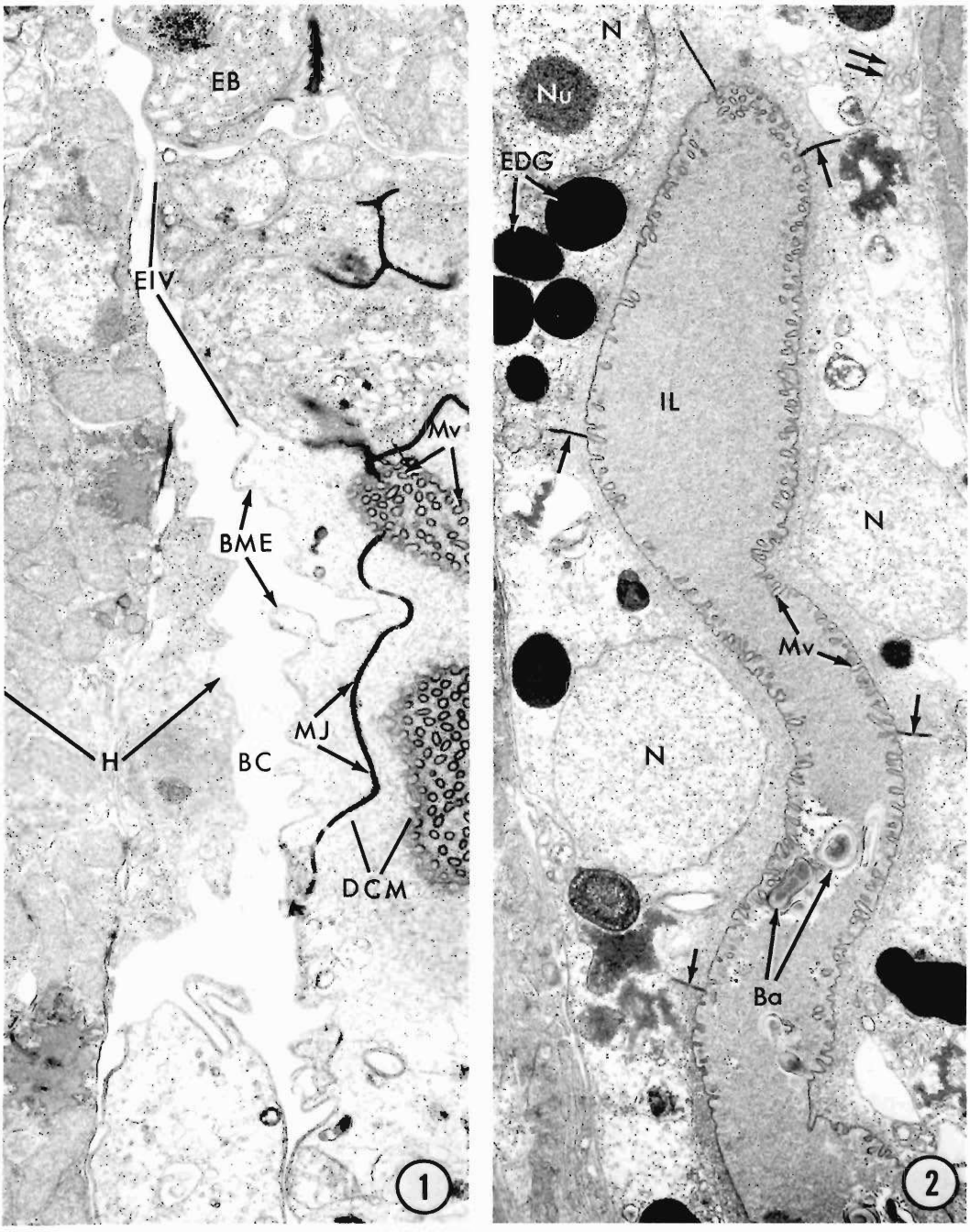

Figures 1, 2. Tangential and mid-longitudinal sections through base of esophagus and intestine of a J3 juvenile of *Heterorhabditis bacteriophora*. 1. Longitudinal–tangential section shows basal boundary of the esophageal bulb (EB) and adjacent 3-tiered cells of the esophago–intestinal valve (EIV). The body cavity (BC) occurs between convoluted basal epithelial membranes (BME) and hypodermis (H). Tangential section through intestine shows base of microvilli (Mv), dense cytoplasmic matrix (DCM), and membrane junctions (MJ). ×11,700. 2. Mid-longitudinal section of mid-region of intestine shows alternate cell arrangements of intestinal epithelium as delineated by nuclei (N), membrane junctions (arrows), and cell membrane appositions (double arrows). The intestinal lumen lined with microvilli (Mv) and containing bacteria (Ba). EDG, electron-dense granules; IL, intestinal lumen; Nu, nucleolus. ×7,190.

mond knife and mounted on uncoated 75 × 300-mesh copper grids. The sections were stained with uranyl acetate and lead citrate and viewed in a Philips 400 electron microscope that was operated at 60 kV with a 20-μm aperture.

Results

Immediately anterior to the intestinal epithelium of *Heterorhabditis bacteriophora* is a tier of cells that forms the esophago–intestinal valve (Fig. 1). These cells are linked to adjacent intestinal cells by clearly defined membrane junctions (Figs. 1, 4). Beyond the membrane junctions, membranes of adjacent cells are in close apposition and follow convoluted pathways to the surface of the epithelium (Fig. 2). Intercellular spaces near the basal surface of the epithelium merge with the body cavity (Figs. 1, 2) which is extensive in the region of the anterior intestinal epithelium and hypodermis. The apical–lateral borders of the epithelium are defined by membrane junctions (Figs. 2, 5, 11). Longitudinal or cross sections of a nematode showed that the cylindrical central lumen of the intestine was lined and supported by a series of paired cells (Figs. 2, 5). These same junctions at the terminus of the apical–lateral positions of paired epithelial cells appeared as elongated membrane junctions when the cells were sectioned tangentially (Fig. 1). Mid-longitudinal sections of the same or similar cells showed a staggered arrangement of epithelial cells that were well defined by the alternate positions of membrane junctions (Figs. 2, 6). The basal portion of the epithelial cells were joined by a labyrinth of interfolded membranes. As the basal–lateral membranes separated, the widened spaces merged with the body cavity (Fig. 9).

Apical membranes of intestinal epithelia have irregular arrangements of microvilli as they evaginate from the anterior surface membranes of the epithelial cells. The microvilli of the anterior to mid-intestinal region were blunt or elongate as the lumen was distended (Figs. 2, 3, 7, 9). In the nondistended sector of the anterior (Fig. 3) and posterior regions of the intestinal epithelium, the microvilli are elongate (Figs. 14, 15). Each microvillus has a few irregularly spaced filaments that extend from its apex to the base. An enteric fibrous coating (EFC) occurs on the surface of microvilli (Fig. 10) and the inter-microvillar surfaces of the apical membranes of the intestinal epithelium (Fig. 9). This coating occurs along the entire length of the intestine, but is variable in density. Integration of microvilli with cellular contents occurs by contact of microvillar filaments with the cellular matrix (Figs. 7, 9, 10). The dense cytoplasmic matrix (DCM) (Figs. 7, 9) of smooth and rough vesicular endoplasmic reticulum occurs adjacent to the intestinal lumen. Each of the intestinal cells appears to have a central prominent nucleus with various concentrations of cytoplasmic inclusions including glycogen, lipid droplets, and granules (Fig. 2). Certain epithelial cells have moderate to dense accumulations of granules with characteristic morphology (Figs. 2, 4, 9, 12). The electron density of these granules varies from uniformly dense to alternating levels of light to dark bands that appear as concentric rings in cross sections (Fig. 12). The intestinal lumen is terminated with a closed cuticularly lined rectum (Fig. 16).

The lumen of the intestine contained populations of bacteria that occurred singly or in large masses within the lumen of the intestine (Figs. 2, 6–8, 13). The rod-shaped bacterial cells were often enclosed or surrounded by membrane-like images (Fig. 13). In certain regions, the lumen appeared clear and the once uniform granular matrix was absent or replaced with spherical to elongate particles (Figs. 6, 13).

Discussion

The anterior limits of the intestine are delineated by the presence of microvilli in the apical membrane of the intestinal epithelium. Closely allied anteriad and adjacent to the intestine was a 3-tiered group of cells. This 3-tiered organ functions as an esophago–intestinal valve because its

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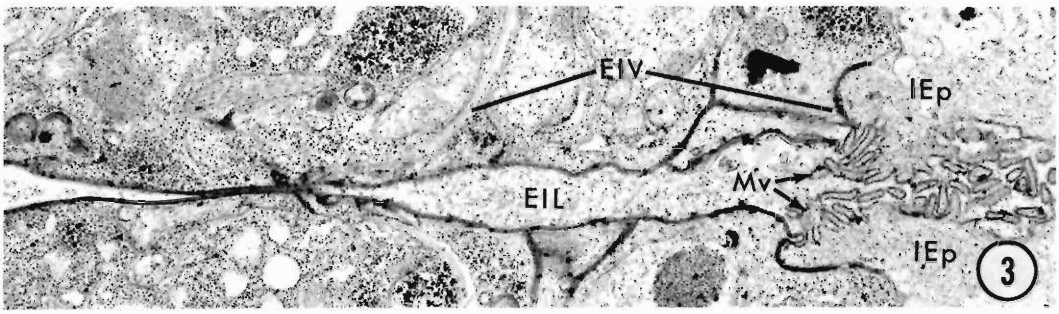
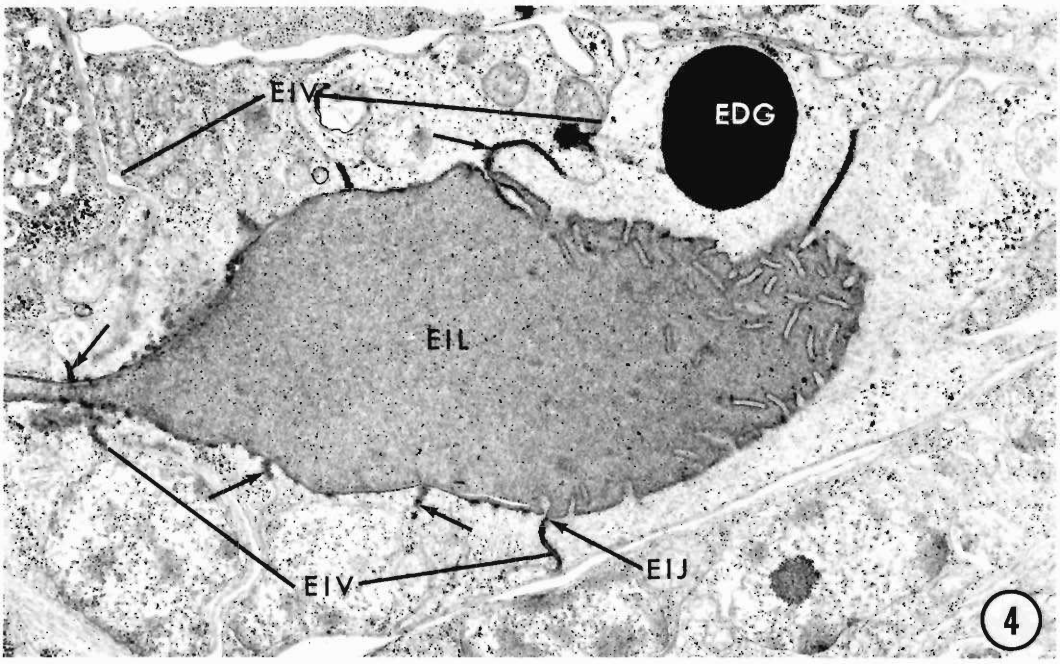
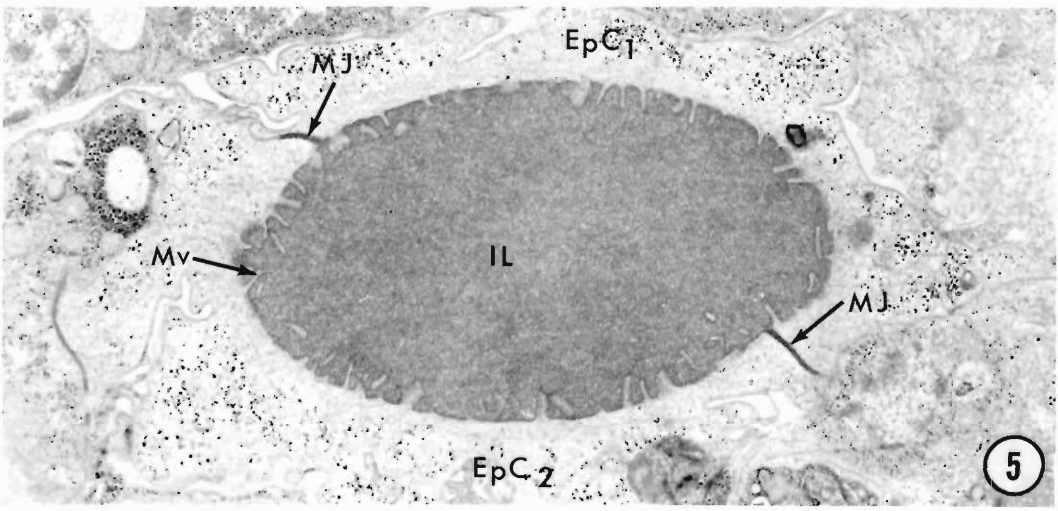
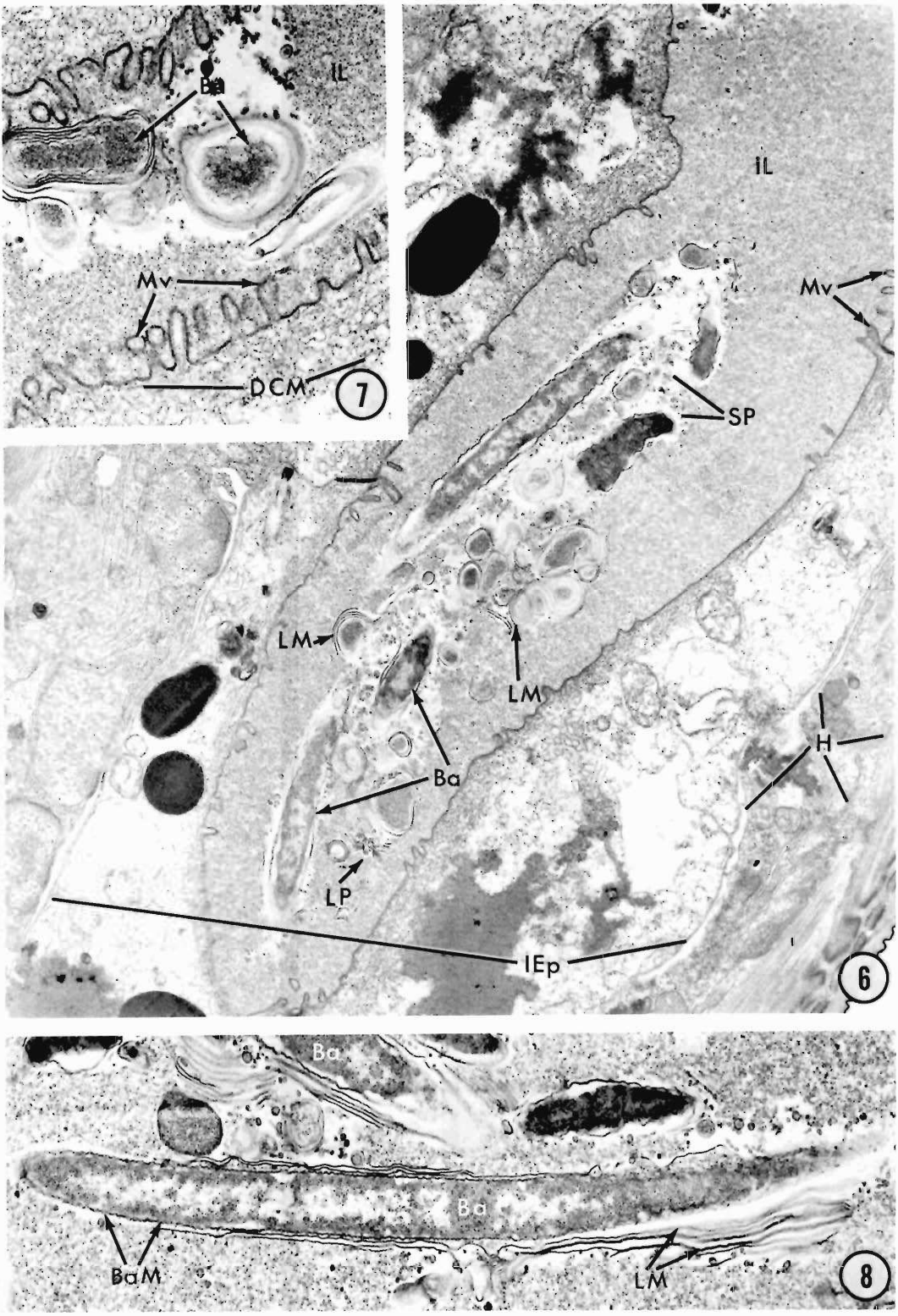
Figures 3–5. Longitudinal–tangential section through esophago–intestinal region and cross section of mid-intestine of *Heterorhabditis bacteriophora*. 3. Longitudinal section showing an esophago–intestinal valve (EIV) in partially closed position with transition to intestinal lumen (EIL) lined by elongated microvilli (Mv) of intestinal epithelium (IEp). ×14,840. 4. Midsection of esophago–intestinal valve (EIV) formed by 3 tiers of cells defined by membrane junctions (arrows) near cuticular wall of lumen. Noncuticularized lumen wall and cell junction delineate beginning of intestinal epithelium with microvilli on apical surface of cells (EIJ). EDG, electron-dense granule; EIL, esophago–intestinal lumen. ×12,460. 5. Cross section of mid-intestine showing paired arrangement of epithelial cells (EpC_1 and EpC_2). IL, intestinal lumen; MJ, membrane junction; Mv, microvilli. ×10,350.

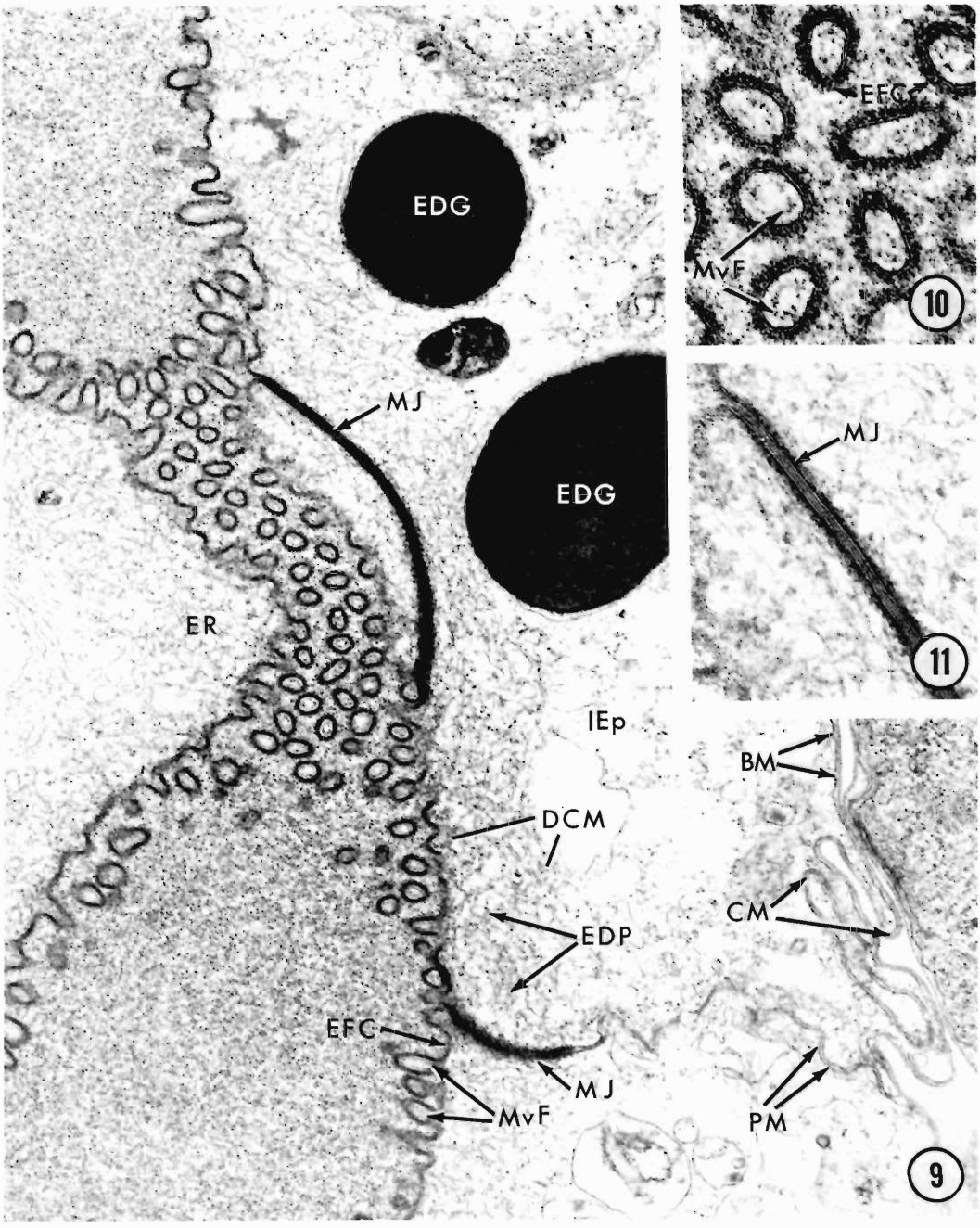








Figures 6–8. Longitudinal sections of mid-region of intestine of infective third stage of *Heterorhabditis bacteriophora*. 6. Broad region of intestinal lumen partially occupied by rod-shaped bacteria (Ba) and associated laminar membranes (LM). Spherical (SP) and linear particles (LP) occur within clear regions of the lumen. *Microvillar* (*Mv*) surfaces are irregular and sparse with portions of the apical membranes devoid of surface evaginations. H, hypodermis; IEp, intestinal epithelium; IL, intestinal lumen. ×10,680. 7. Anteriad region of



Figures 9–11. Longitudinal mid- to tangential sections of intestine of *Heterorhabditis bacteriophora*. **9.** A portion of epithelial cell (IEp) with electron-dense granules (EDG) with apical membrane surfaces lined with endoplasmic reticulum (ER) and electron-dense particles (EDP). Microvilli contain longitudinally oriented filaments (MvF). Adjacent epithelial cells are joined near lumen with membrane junctions (MJ). Paired membrane (PM) closely apposed laterally and convoluted (CM) especially near the basal membrane (BM). DCM, dense cytoplasmic matrix; EFC, enteric fibrous coat. ×27,150. **10.** Enlargement of portion of microvilli showing irregular arrangements of filaments (MvF) and trilaminar membranes of microvilli. EFC, enteric fibrous coat. ×107,360. **11.** Enlargement of membrane junction (MJ) of Figure 9. Note zone of cell membrane apposition in which an intercellular space is evident, uniform in width, and filled with electron-dense material. ×70,960.

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intestine of Figure 6 shows a narrowing of the lumen, bordered by vesicular endoplasmic reticulum as part of dense cytoplasmic matrix (DCM), high number of microvilli (Mv), and apical cell membranes. Ba, bacterial cells; IL, intestinal lumen. ×24,000. **8.** Section through intestinal lumen showing a bacterium (Ba) with a distinct cell wall membrane. BaM, bacterial membrane; LM, lamellar membrane. ×18,240.

cuticular lumen is continuous with the broad intestinal lumen.

Longitudinal sections through the intestine of *H. bacteriophora* show the moderate number of nucleated cells that comprise the intestinal epithelium. This species follows the oligocytous arrangement of intestinal epithelial cells consisting of fewer than 128 cells per organ (Chitwood and Chitwood, 1950; Bird, 1971). In contrast, *Ascaris suum* has a myriocytous arrangement that exceeds 8,000 cells per specimen. Although the numbers of cells comprising intestinal epithelia vary widely among nematodes, the presence of microvilli and the terminal web are common features. The inner core of the microvilli of *A. suum* has elongated fibers that extend for short distances into the epithelial cell and terminate in a subbacillary layer. This layer, with adjacent fibrous network and small granules, forms the terminal web (Sheffield, 1964). The filaments in the central core of the microvilli in *H. bacteriophora* were similar but less numerous than those described for *A. suum*. The dense cellular matrix at the bases of microvilli appear not to be a terminal web in the form discussed by Munn and Greenwood (1984) but may be a region of intestinal cell secretion and absorption related to the digestive functions of the nematode. The membrane junctions of adjacent intestinal epithelial cells near the lumen appeared to form a continuous belt-like junction with a mechanism to retain intestinal cell and lumen continuity. Similar cell junctions occur between epithelial cells of higher animals (Fawcett, 1966).

The enteric coating on the microvilli and intermicrovillar surface of the apical membranes of the intestinal epithelium is similar to the coating that occurs on the apical membranes of intestines of *Aphelenchoides blastophthorus* (Shepherd et al., 1980) and of *Heterodera glycines* (Endo, 1988) but lack the highly sculptured appearance of the latter species. Additional research is needed to determine the nature of the enteric material and its possible role in food uptake and digestion among plant and insect–parasitic species.

The granules with concentric rings of electron density present in the intestinal epithelial cells of *H. bacteriophora* were quite distinct from liquid droplets. The granules also occurred in insects and appeared like glycolipids or lipoproteins that had been partially degraded and gave a differential stain reaction. However, cytochemical analyses will be necessary to elucidate the true nature of these particles. In terms of the fine structure, abundance, and general presence of granules in the intestinal epithelia, the granules may be similar to the particles described in rhabditoids using the light microscope. They may constitute the birefringent-sphearocrystals that were observed in rhabditoid species (Cobb, 1914, Jacobs and Chitwood, 1937). The sphearocrystals of the intestines were reported as gray in color, bright spots in dark field illumination, and bright spots with a central cross when observed with polarized light. Similar dense bodies were observed within the midgut of the housefly, *Musca domestica.* It is suggested that mineralized dense bodies are sites of intercellular sequestration of minerals and may play a vital functional role in the excretory system (Sohal et al., 1977). Among lepidopterous larvae, dense bodies within the midgut take on a laminated appearance and are termed spherites. They appear first before ecdysis and disappear during differentiation of regenerative cells to columnar and goblet cells (Turbeck, 1974).

Physiological studies were not used to identify the bacteria observed in the lumen of *H. bacteriophora* of this study. However, the nematodes were samples taken from cultures previously used for infectivity studies that were consistent with observations of Poinar et al. (1977) in which the bacteria were identified as *X. luminescens* (Thomas and Poinar, 1979). They were able to liberate bacteria from the pharynges and intestines of surface sterilized juveniles of infective J3 of *H. bacteriophora* by placing the nematodes in a drop of insect blood. It is evident that when the J3 juveniles enter an insect host, and penetrate the body cavity, bacteria are released into the insect hemolymph. The bacteria multiply

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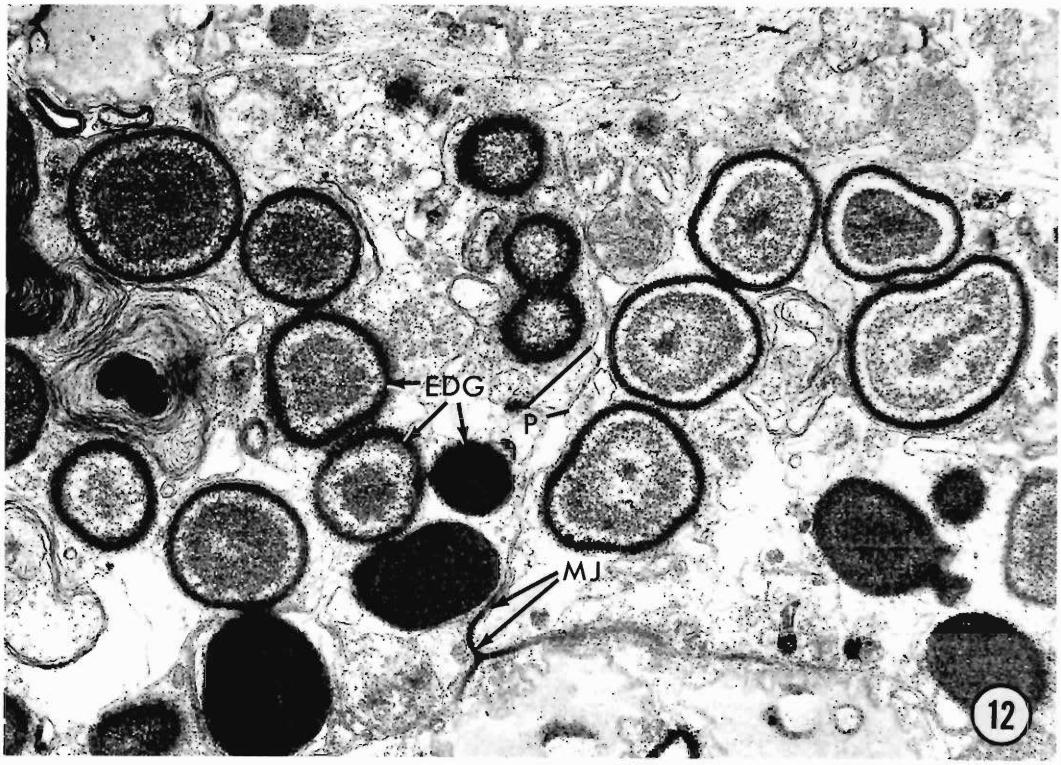
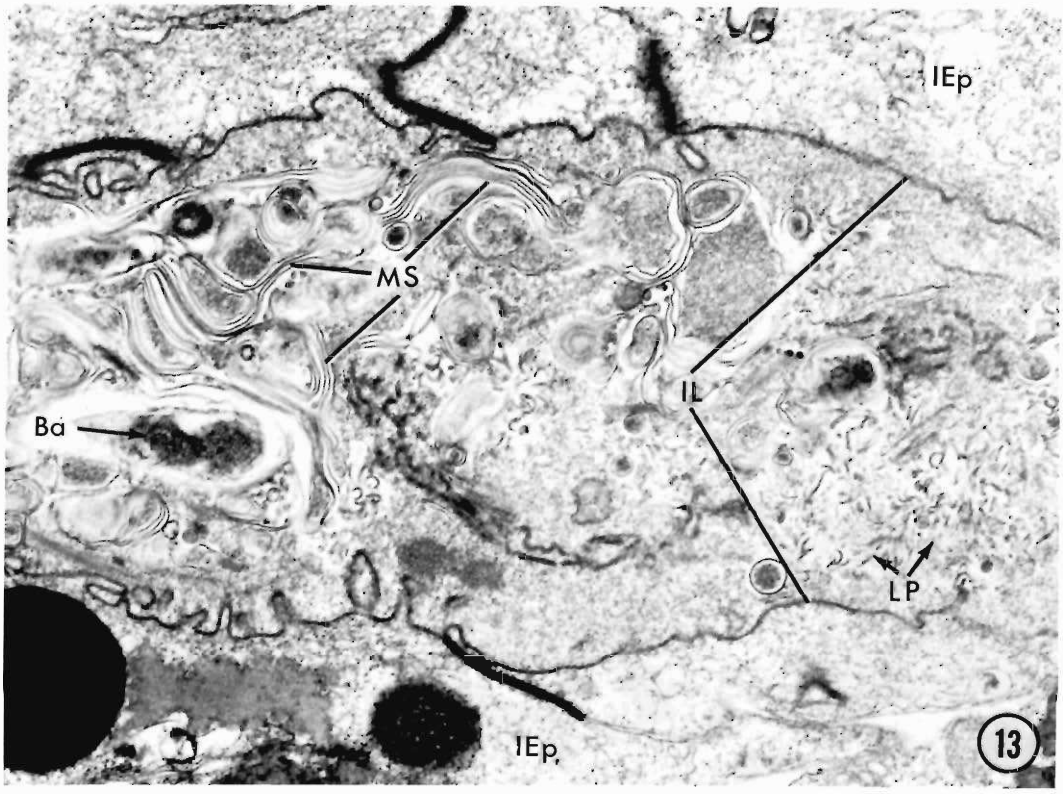
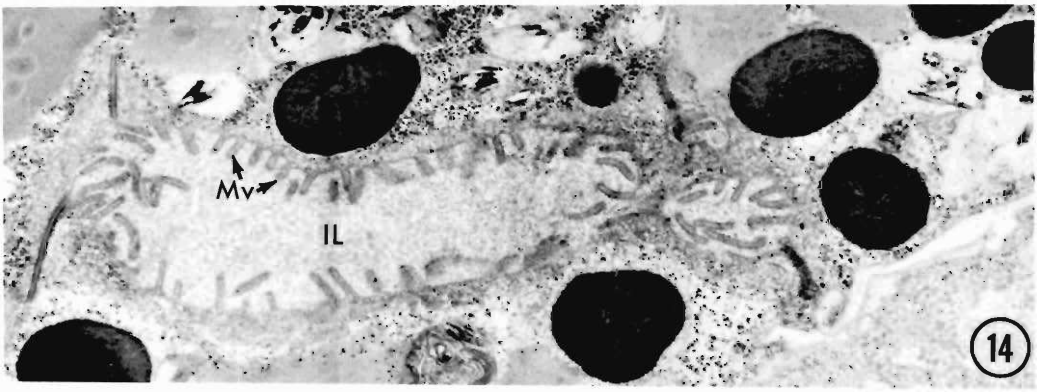
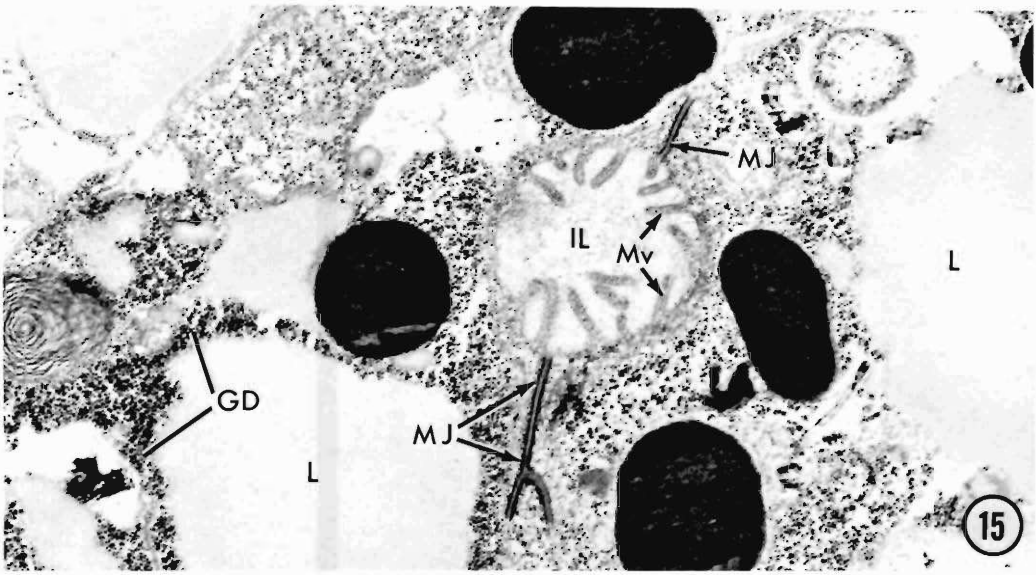
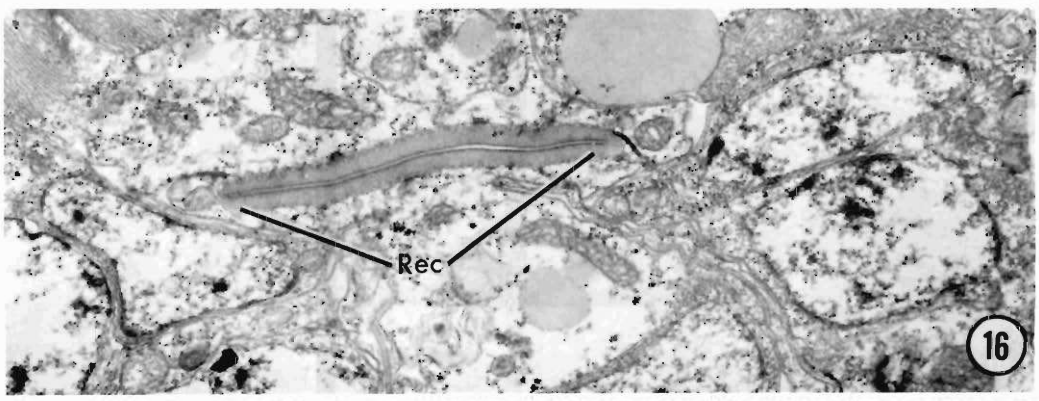
Figures 12, 13. Longitudinal sections through intestinal epithelial cell and lumen of intestine showing granules and bacteria, respectively. 12. Median section through portions of 2 epithelial cells with granules (EDG) of varying degrees of electron density and size; appearance of concentric rings of stain apparently dependent on sectioning levels through each spheroid granule. P, plasmalemmae; MJ, membrane junctions. ×14,180. 13. Mid-longitudinal section through lumen of intestine (IL) with bacterium (Ba) and associated membrane swirls (MS) and linear particles (LP). Number of microvilli evaginated from the apical cell surface are variable; microvilli may be absent along portions of lumen surface. IEp, intestinal epithelium. ×21,600.











Figures 14–16. Longitudinal and cross sections through posterior region of intestinal epithelium and cuticularized rectum. **14.** Longitudinal–tangential section of posterior intestinal lumen (IL) lined with microvilli (Mv). Epithelial cells contain prominent lipid droplets and granules. ×11,590. **15.** Cross section of a different nematode showing a circular form of intestinal lumen (IL) lined with microvilli (Mv). Epithelial and hypodermal sections have large lipid droplets (L), some surrounded by dense glycogen deposits (GD). MJ, membrane junctions. ×18,240. **16.** Cross section of tail region of *Heterorhabditis* shows cuticle lined rectum (Rec) and supporting cells. ×14,350.

rapidly, and their large populations become toxic to the insect and cause septicemia. In a related study, Poinar (1966) showed that *Achromobacter nematophilus* was contained in the ventricular region of the intestinal lumen of *Steinernema carpocapsae,* strain DD 136. In addition, bacteria were found in the epithelial cells of the intestine. In our study, bacteria were not observed in epithelial cells of *H. bacteriophora.*

Future work should focus on changes in bacterial morphology and multiplication in the intestinal lumen during the transition of the infective J3 nematode from the nonfeeding stage to the feeding stage within the insect host. Observations should be made to determine the nature and accumulation of bacteria-associated membrane-like particles that may be products of autolysis or lytic action of bacteria on the lumen contents. Ultimately, it may be possible to correlate the fine structure observations of the bacteria in nematodes with the effectiveness of species or strains of rhabditoid nematodes as biological control agents against important agricultural insect pests.

Acknowledgments

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ANNOUNCEMENT

Ostertagia Workshop 1–4 March 1992

A 2½-day workshop on *Ostertagia* will be held 1–4 March 1992 at the University of Maryland, University College Center of Adult Education, College Park, Maryland. The scientific program is intended to be comprehensive on all aspects of research related to *Ostertagia ostertagi* and diseases associated with this parasite. The workshop will include invited speakers and roundtable discussions on topics to promote research and control. These include systematics, epidemiology, immunity, modeling, genetics, chemotherapy, diagnosis, and effects on host physiology. A poster session will be included in the program as an evening session to allow interested participants an opportunity to display current research from their laboratories related to ostertagiosis. Participation will be limited. Prompt registration is suggested.

For further information about the workshop, agenda, and scientific sessions, please call or write: Dr. Daniel E. Snyder, USDA, ARS, Animal Parasite Research Laboratory, P.O. Box 952, Auburn, Alabama 36831; Telephone (205) 887-3741; FAX (205) 821-1732.